Abstract

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Abstract: DESCRIPTION (provided by applicant): We propose to develop an HTS assay at the University of New Mexico, Albuquerque (Larry Sklar, PI), based on a new regulatory phenomenon recently discovered by my research group, the controlled disassembly of the 26S proteasome in the presence of endogenous proteasome-interacting proteins (Babbitt et al. 2005, Cell: 121, 553-565). Ubiquitin-mediated protein degradation by the proteasome has only recently been recognized as critical for cell growth and proliferation. Already, perturbations of this system have been implicated in multiple aspects of cancer pathogenesis, marking the proteasome as an attractive therapeutical target. The project I propose here stems from my long-term goal to understand the molecular mechanisms by which the proteasome recruits substrates and facilitates their destruction. My research group addresses this goal via biochemical dissection of protein degradation in vitro, using purified substrates and components of the SCF ubiquitin ligase pathway of yeast S. cerevisiae, which we reconstructed in vitro and characterized (Skowyra et al. 1997, Cell: 91, 209-219; Skowyra et al. 1999, Science: 284, 662-665; Kamura et al. 1999, Science: 284, 657-661; Deffenbaugh et al. 2003, Cell: 114, 611-622; Babbitt et al. 2005, Cell: 121, 553-565). This pathway is conserved and controls the degradation of major G1 cell cycle regulatory proteins and signaling molecules in all organisms, be they yeast or human. The information obtained with yeast is therefore directly relevant and, frequently, key to understanding SCF-mediated proteolysis in human cells. In our studies, we seek to uncover features of the proteasome that could serve as targets for pharmacological regulation of its activity at the steps of substrate recognition and processing for degradation, but not degradation itself. This knowledge will likely prove of considerable significance for the development of novel strategies for targeting the proteasome in cancer and other diseases linked to abnormal protein degradation. In collaboration with Larry Sklar, we have developed several flow cytometry-based assays for monitoring the rate-limiting, regulatory steps in the SCF-proteasome pathway (Deffenbaugh et al. 2003, Cell: 114, 611- 622; Babbitt et al. 2005, Cell: 121, 553-565; and data unpublished). The assay proposed here will allow us to identify, in a single screen, a collection of small molecules that could interfere with the catalytic cycle of the proteasome via a variety of mechanisms. Subsequent analysis of these molecules in the multiple in vitro assays available in my laboratory will allow us to identify the precise mechanisms by which the compounds function. As I explain below, such molecules are likely to become an invaluable research and therapeutical tool.

Thesaurus Terms:
High throughput screening, 26S proteasome, proteasome-interacting proteins, Ubiquitin, cancer pathogenesis, protein degradation in vitro, SCF ubiquitin ligase pathway, yeast S. cerevisiae, G1, SCF-mediated proteolysis, flow cytometry, ATP Hydrolysis

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